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Distribution of micro-organisms along a transect in the South-East Pacific Ocean (BIOSOPE cruise) from epifluorescence microscopy

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Abstract

The distribution of selected groups of micro-organisms was analyzed along a South-East Pacific Ocean transect sampled during the BIOSOPE cruise in 2004. The transect could be divided into four regions of contrasted trophic status: a high Nutrient Low Chlorophyll (HNLC) region (mesotrophic) near the equator, the South-East Pacific Ocean gyre (hyper-oligotrophic), the transition region between the gyre and the coast of South America (moderately oligotrophic), and the Chile upwelling (eutrophic). The abundance of phycoerythrin containing picocyanobacteria, autotrophic and heterotrophic eukaryotes in different size ranges, dinoflagellates, and ciliates was determined by epifluorescence microscopy after DAPI staining. All populations reached a maximum in the Chile upwelling and a minimum near the centre of the gyre. Picocyanobacteria reached a maximum abundance of 70×10^3 cell mL^{-1}. In the HNLC zone, up to 50% of picocyanobacteria formed colonies. Autotrophic eukaryote and dinoflagellate abundance reached 24.5×10^3 and 200 cell mL^{-1}, respectively. We observed a shift in the size distribution of autotrophic eukaryotes from 2–5 μm in eutrophic and mesotrophic regions to less than 2 μm in the central region. The contribution of autotrophic eukaryotes to total eukaryotes was the lowest in the central gyre. Maximum concentration of ciliates (18 cell ml^{-1}) also occurred in the Chile upwelling, but, in contrast to the other groups, their abundance was very low in the HNLC zone and near the Marquesas Islands.

1 Introduction

Unicellular picoplanktonic prokaryotes and eukaryotes less than 2 μm in size (Sieburth et al., 1978) are found in marine ecosystems at concentrations ranging from 10^2 to 10^5 and 10^2 to 10^4 cells mL^{-1}, respectively. They play a fundamental role (Azam et al., 1983; Sherr and Sherr, 2000), in particular, in oligotrophic waters (Hagström et al., 1988; Maranon et al., 2001) where their small size associated to the reduced diffusion

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boundary layer and large surface area per unit volume are an advantage to acquire nutrients (Raven, 1998). The photosynthetic component of picoplankton, i.e. *Prochlorococcus* and *Synechococcus* cyanobacteria and picoeukaryotic algae, are important contributors to the microbial community of the euphotic zone in many marine environments (Campbell et al., 1997; Mackey et al., 2002; Perez et al., 2006). Heterotrophic protists play a pivotal role in mediating organic flux to higher trophic levels in pelagic ecosystems (Azam et al., 1983; Fenchel, 1982; Hagström et al., 1988). Among the heterotrophic protists, ciliates and dinoflagellates are potentially important grazers of picoplankton (Sherr and Sherr, 2000).

In the Pacific Ocean, picoplankton has been analyzed both in the Equatorial region and the North gyre (e.g. Campbell et al., 1997; Mackey et al., 2002) but not in the South gyre. The latter is the most oligotrophic environment of the world oceans based on SeaWifs imagery which provides estimates of average surface chlorophyll *a* concentrations down to 0.02 mg m^{-3} (Morel et al., 2007).

The BIOSOPE (Biogeochemistry & Optics South Pacific Experiment) cruise explored this region sailing from the Marquesas Islands to the coast of Chile. Along this transect, a gradient in trophic conditions was encountered, from hyper-oligotrophic (gyre) to very eutrophic waters (Chile upwelling). The present study focuses on the distribution of phycoerythrin containing picocyanobacteria, autotrophic and heterotrophic eukaryotes (in particular dinoflagellates and ciliates) in the South-East Pacific as estimated by epifluorescence microscopy. In comparison to more rapid enumeration techniques such as flow cytometry, microscopy allows (1) to discriminate specific group of organisms such as dinoflagellates, (2) to recognize cell organization such as colonies and (3) to regroup organism into size classes (e.g. here, smaller than 2 μm, between 2 μm and 5 μm, and larger than 5 μm). In this paper we try to relate the distribution of the different types of organisms to oceanographic conditions and in particular trophic status.

2 Material and methods

2.1 Oceanographic context

The BIOSOPE cruise took place on board the French NO "l'Atalante" in the South East Pacific Ocean from 26 October to 11 December 2004 (Fig. 1). The transect investigated extended from the Marquesas Islands (South Pacific Tropical Waters; SPTW) to the coast of Chile, through the Eastern South Pacific Central Waters (ESPCW) which include the centre of the Pacific gyre (Claustre et al., 2007[1]). The transect can be divided into four very contrasted trophic zones (from West to East): a high Nutrient Low Chlorophyll (HNLC) zone (mesotrophic) near the equator, the South-East Pacific gyre (hyper-oligotrophic) itself, the transition zone between the gyre and the coastal region (moderately oligotrohic), and the Chile upwelling (very eutrophic). In the hyper oligotrophic zone, nitrate concentrations were nearly undetectable between the surface and 150–200 m and remained very low (~2.5 μM) below this depth (Fig. 2b). Nitrate concentrations were higher in the HNLC zone and maximum in the Chile upwelling (Fig. 2b, Raimbault and Garcia, 2007[2]). Phosphate was apparently never a limiting factor (Fig. 2c, Moutin et al., 2007[3]).

[1] Claustre, H., Sciandra, A., Vaulot, D., and Raimbault, P.: Introduction to the special section : bio-optical and biogeochemical conditions in the South East Pacific in late 2004 – the BIOSOPE program, Biogeosci. Discuss., in preparation, 2007.

[2] Raimbault, P. and Garcia, N.: Nutrients distribution and new production estimation in the southwest Pacific: Evidence for intense nitrogen recycling, Biogeosci., Discuss., submitted, 2007.

[3] Moutin, T., Karl, D., Duhamel, S., Rimmelin, P., Van Mooy, B., and Claustre, H.: Phosphate availability and the ultimate control of nitrate input by nitrogen fixation in the Pacific Ocean, Biogeosci. Discuss., submitted, 2007.

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2.2 DAPI staining and epifluorescence microscopy

Fifteen stations (Fig. 1 and Table 1) were sampled at six depths with a conductivity-temperature-depth (CTD) rosette system equipped with 12 L Niskin bottles. In general, two samples were collected in the surface layer, three around the chlorophyll maximum and one below. Water was pre-filtered through a 200 μm mesh to remove zooplankton, large phytoplankton, and particles before further filtrations.

Water samples (100 mL) were fixed with glutaraldehyde (0.25% final concentration) and filtered through 0.8 μm pore size filters. This porosity was selected to avoid high densities of bacteria on the filter which would have rendered visualisation of the larger and less dense eukaryotes more difficult. Samples were stained with 4'6-diamidino-2-phenylindole (DAPI, 5 μg mL^{-1} final concentration) (Porter and Feig, 1980) and stored at –20°C for at least 12 months before counting. Counts were performed with an Olympus BX51 epifluorescence microscope (Olympus Optical CO, Tokyo, Japan) equipped with a mercury light source and an x100 UVFL objective. Some pictures, such as those of dinoflagellates, were taken on board the ship on the freshly prepared slides using a BH2 Olympus microscope with an x40 objective and a Canon G5 digital camera. Pictures of picocyanobacteria were taken in the laboratory on the BX51 Olympus microscope with a Spot RT-slider camera (Diagnostics Instruments, Sterling Heights, MI).

Isolated and colonial picocyanobacteria (Fig. 3) containing orange fluorescing phycoerythrin were counted under green light (530–550 nm). DAPI staining allowed us to discriminate eukaryotic from prokaryotic organisms under UV light (360/420 nm) based on the blue staining of the cell nucleus. The red fluorescence of chlorophyll under blue light (490/515 nm) allowed us to discriminate autotrophic (photosynthetic) from heterotrophic eukaryotes. However, it was not possible to distinguish truly autotrophic organisms from organisms that had ingested chlorophyll-containing cells. Ten fields and a minimum of 100 cells were counted per slide. Eukaryotes were classified according to three diameter ranges: (i) smaller than 2 μm, (ii) between 2 μm and 5 μm,

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(iii) larger than 5 μm. Among eukaryotes larger than 5 μm, ciliates and dinoflagellates were counted separately. Dinoflagellates were discriminated by their shape, their size (between 5 μm and 100 μm), and the presence of a nucleus with condensed chromatin. Within dinoflagellates, we discriminated autotrophic and heterotrophic dinoflagellates based on the red fluorescence of chlorophyll under blue light of the former (Fig. 4a and b). Among heterotrophic dinoflagellates, some dinoflagellates were characterized by an intense green fluorescence under blue light (Fig. 4c), as reported previously (Shapiro et al., 1989), and counted separately. Ciliates were discriminated by their shape, their size (between 20 μm and 100 μm), and the presence of cilia and multiple nuclei. No distinction between different types of ciliates was attempted. Because of their low abundance, 50 fields per slide were counted for dinoflagellates and ciliates such that the minimum concentration detectable was 1.5 cell mL^{-1}.

2.3 Data representation

Contour maps showing the distributions of CTD data and of the different populations were drawn using the Ocean Data View software (Schlitzer, 2003). VG gridding with averaging length-scales of 45 and 100 for both X and Y was used for CTD and microscopy data, respectively.

3 Results

3.1 Comparison between microscopy and flow cytometry

In order to validate our microscopy counts, we compared them to counts of *Synechococcus* cyanobacteria and photosynthetic picoeukaryotes done by flow cytometry (Grob et al., 2007) at the same stations (Fig. 5). There was a good relatively correlation between the two methods, such that global distribution trends were identical, but slopes were significantly larger than one indicating that microscopy was underestimating the

actual concentrations. For picocyanobacteria, abundance found by microscopy was 3 times lower than measured by flow cytometry (Fig. 5a). For photosynthetic eukaryotes, the correlation was not very high (R^2=0.69; n=80) when all the data were considered, although the slope was lower than for cyanobacteria (Fig. 5b). However, if only data below 40–60 m were included the correlation was significantly better (R^2=0.90; n=56) and the slope less pronounced.

3.2 Picocyanobacteria

In surface, picocyanobacteria abundance (Fig. 6a) reached a maximum (70×10^3 cell mL^{-1}) near the coast of Chile (station UPW1) and a minimum (less than 500 cell mL^{-1}) in the middle of the South East Pacific gyre. They increased again near the Marquesas Islands. Along the vertical, they decreased slightly down to about 100 m and then quickly disappeared (Fig. 6a). Interestingly, a large fraction of the picocyanobacteria belonged to colonial forms in the vicinity of the Marquesas Islands and in the HNLC zone (Fig. 6b). In this region, this fraction could reach up to 50% percent near the surface and 5 to 10% between 25 and 100 m, while it dropped below 5% almost everywhere else. Three types of colony could be observed (Fig. 3): (i) groups of 20–30 cells, (ii) groups of more than 100 cells, (iii) short chain. None of these forms seemed to be preferentially observed in any given region.

3.3 Eukaryotes

The maximum abundance of total eukaryotes (26×10^3 cell mL^{-1}) occurred in the Chile upwelling near the surface (station UPX2, 25 m depth) and the minimum (276 cell mL^{-1}) in the gyre at depth (station GYR2, 270 m depth) (Fig. 7a). In the surface layer, abundances were minimum in the center of the gyre and increased both eastward and westward. The maxima of total eukaryotes coincided roughly with the depth of chlorophyll maximum (DCM) (Compare Figs. 2a and 7a). Below 200 m, concentrations were always lower than 1000 cell mL^{-1}. The distribution of autotrophic eukaryotes was very

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similar to that of total eukaryotes (Fig. 7b), a consequence of the fact that they were much more abundant than heterotrophic ones (Fig. 7c). The size distribution of autotrophic eukaryotes varied dramatically throughout the transect (Figs. 8 and 9): in the surface of the gyre, cells smaller than 2 μm accounted for less than 10% while, they dominated (50–70 %) in the DCM of the gyre as well as east of the gyre (Fig. 8A). In the Chile upwelling (station UPX2, 25 m), they accounted for up to 80% of the total eukaryotes. In contrast, their contribution was much lower in the HNLC region where larger eukaryotes between 2 μm and 5 μm accounted for 40% to 60% of the population (Fig. 8b). This size class was also dominant near the surface in the transition zone between the gyre and the upwelling. Cells larger than 5 μm accounted for less than 10% of autotrophic eukaryotes everywhere along the transect, except near the Marquesas Islands where they contributed slightly more (Fig. 8c).

The relative proportion of heterotrophic eukaryotes was the highest in the 0–50 m layer of the gyre (75–80%), while in the DCM it dropped to 25% (Fig. 7c). In the DCM, cells smaller than 2 μm accounted for 28% (east of the gyre) to 40% (in the gyre) of heterotrophic eukaryotes (Fig. 9). The contribution of cells between 2 μm and 5 μm did not vary much (about 50 %) while cells larger than 5 μm accounted for up to 14% of total heterotrophic eukaryotes in the HNLC region and for about 10% elsewhere.

In the 0–100 m layer, dinoflagellate abundance (Fig. 10a) increased towards the HNLC region (maximum observed: 200 cell mL^{-1} at station STB1, 25 m) and the Chile upwelling, and decreased towards the gyre (minimum observed: 10 cell mL^{-1} at station GYR2, 270 m) in the gyre. In relative terms, autotrophic dinoflagellates dominated around the Marquesas Islands (up to 80% of total dinoflagellates, at station STB1, 80 m depth) and in the Chile upwelling (70% at station UPW1, 15 m depth) (Fig. 10b). The maximum of percentage of autotrophic dinoflagellates (50%–80%) followed the DCM except at station STB8 where the highest percentage (50%) occurred at 70 m whereas the DCM was found at 170 m (Fig. 2a and 10b). In the Chile upwelling, the maximum of autotrophic dinoflagellates (50% at station UPX2 in surface and 70% at station UPW1 at 15 m) occurred above the DCM. The percentage of autotrophic dinoflagellates was

the lowest (5%–25%) in the surface of the gyre and below 250 m.

Heterotrophic dinoflagellates contribution ranged from 20% to 95% of the total (Fig. 10c) and consisted mostly (80% on average) of cells smaller than 20 μm in size (data not shown). Vertical profiles showed that maximum abundances of heterotrophic dinoflagellates followed the DCM only at some stations in the gyre (STB3, STB6 and STB8, Fig. 11). At other stations, the maximum abundance of heterotrophic dinoflagellates was observed above the DCM, except in the upwelling (station UPX2) where the maximum was found below. At station EGY2 (east of gyre), the lowest concentration of heterotrophic dinoflagellates (18 cell mL^{-1}) occurred in the DCM.

Green fluorescing dinoflagellates (Fig. 4c) accounted for up to 50% of the heterotrophic dinoflagellates in surface east of the gyre and at depth in the Chile upwelling and for 5 to 25% of heterotrophic dinoflagellates in the HNLC zone and the Chile upwelling (Fig. 10d).

Ciliates abundance reached a maximum (18 cell mL^{-1}) in the Chile upwelling (station UPW1, 40 m depth) and a minimum in the HNLC region (Fig. 12). Abundance increased towards the Chile upwelling and decreased towards the gyre as for most other groups. However, in contrast to most other groups, ciliates also remained quite low towards the HNLC zone and the Marquesas Islands. Vertically, at many stations, ciliate maxima corresponded to dinoflagellate minima (Fig. 11).

4 Discussion

Difference between abundances estimates by microscopy vs. flow cytometry observed in this study could be due to several reasons. First, some cells smaller than could have passed through the 0.8 μm filter used here. If this is a real possibility for picocyanobacteria, the loss of eukaryotic cells is likely to be negligible since the smallest known eukaryote *Ostreococcus tauri* has a size of 0.8 μm (Courties et al., 1994). This may explain why the slope is larger for cyanobacteria than eukaryotes (Fig. 5). Second, samples for microscopy were stored for more than a year at –20°C before

counting while samples for flow cytometry were analyzed fresh on board. Gundersen et al. (1996) and Putland et al. (1999) showed a significant loss of bacteria after one month and unicellular cyanobacteria after three months storage of samples at –20°C, respectively. Third, storage at –20°C may cause a degradation of chlorophyll and an underestimation of red fluorescing organisms (Chavez et al., 1990). Therefore, abundances of unicellular picocyanobacteria and autotrophic eukaryotes may be underestimated, while the proportion of heterotrophic eukaryotes could be higher than in the initial samples. The discrepancy between the correlation slopes for the different populations (Fig. 5) could reinforce this hypothesis. In fact, we observed during counting that organisms from surface samples had less intense chlorophyll fluorescence than those of deeper samples (as expected due to photoacclimation), but also that fluorescence fading was faster. Therefore, it was not always easy to distinguish autotrophic organisms from heterotrophic organisms near the surface, which could explain the lower correlation and higher slopes between abundances for samples above 40–60 m.

The low abundance of unicellular picocyanobacteria containing phycoerythrin in the gyre and their higher abundance in the Chile upwelling, a region rich in nutrients and characterized by mixed waters, is in agreement with many recent studies (for a review see Partensky et al., 1999). Interestingly, up to 50% of the unicellular picocyanobacteria appeared to be colonial near the Marquesas Islands and in the HNLC region (Fig. 3 and 6c). Some cyanobacteria encountered in marine systems form colonies (Graham and Wilcox, 2000) but these are usually much larger than those we observed. It is, for example, the case for *Trichodesmium* which have been previously observed in the Equatorial Pacific (Capone et al., 1997). Interestingly, unicellular picocyanobacteria forming chains (cf. Fig. 3d) were isolated in culture from the HNLC station at 30 m and 100 m depth (Le Gall et al., 2007), but the other morphotypes observed in the field were not obtained. Since there were some evidence of nitrogen fixation activity in this area (P. Raimbault, personal communication), it is tempting to hypothesize that these colonial picocyanobacteria could be nitrogen-fixing. However, small cyanobacteria re-

cently shown to have the capacity to fix nitrogen (Zehr et al., 2001) do not seem to form colonies and their morphotype (spherical 3–10 μm cells) has been rarely observed in our samples (data not shown). Alternatively, colony formation could be an adaptation to the structure of the predator community in this region, such as the higher the dinoflagellate to ciliate ratio. Indeed, cells forming colonies could take advantage of the positive aspects of increased size, in particular lower grazing pressure, without paying the full cost of decreased metabolism and reduced growth which is associated with large individual cell size (Nielsen, 2006).

Globally, the distribution of picophytoplankton and nanophytoplankton obtained in the present study is consistent with that estimated by Ras et al. (2007)[4] from HPLC pigment data based on assumptions concerning the size range of specific taxonomic groups (Claustre, 1994; Vidussi et al., 2001). They found that the contribution of picophytoplankton (in terms of percentage of Tchl*a*) was the highest in the gyre itself and east of gyre, while nanophytoplankton dominated in the HNLC zone and the Chile upwelling. However, their method tends to underestimate the contribution of picophytoplankton and to overestimate the contribution of macrophytoplankton. For example, they only took into account for the picoplankton size group pigments characterizing cyanobacteria and Chlorophyta. However, Prymnesiophyceae may also contribute significantly to picoeukaryotic population (Moon-van der Staay et al., 2000; Not et al., 2005). Indeed, Prymnesiophyceae cells characterized by two chloroplasts were observed in our DAPI samples (data not shown). Conversely, Ras et al. (submitted) include pigments of dinoflagellates and diatoms in the microplankton size range (20–200 μm), while many dinoflagellates and some diatoms smaller than 20 μm (data not shown) have been observed along the South-East Pacific transect, as observed previously along 110° W. (Hardy et al., 1996). Therefore, the contribution of microphytoplankton could be overestimated.

Grob et al. (2007) demonstrated that autotrophic picoeukaryotes are important con-

[4]Ras, J., Uitz, J., and Claustre, H.: Spatial variability of phytoplankton pigment distribution in the South East Pacific, Biogeosci. Discuss., in preparation, 2007.

tributors to the photosynthetic carbon biomass in the gyre. As a complement, we show here that heterotrophic eukaryotes account for up to 90% of total eukaryotes in the gyreand that between 20 and 65% of them are picoplanktonic in size. Therefore the total contribution of picoeukaryotes (autotrophic and heterotrophic together) to particulate carbon biomass is probably very significant.

During the BIOSOPE cruise, Gomez et al. (2007) found dinoflagellate abundance always lower than 1 cell mL^{-1}, except at station station 20 where a bloom of dinoflagellates was observed (~4 cells mL^{-1} between surface and 5 m depth), and at station UPW (~2 cells mL^{-1}). These counts from acidified lugol's fixed samples are much lower than ours (Table 1). These differences could originate from the size of counted dinoflagellates in both studies. We counted dinoflagellates which were between 5 μm and 50 μm in diameter while Gomez et al. (2007) only counted dinoflagellates larger than 15 μm. Hardy et al. (1996) showed that dinoflagellates larger than 20 μm accounted only for 10 to 30% of total dinoflagellates in the Pacific gyre. In our samples (data not shown), the contribution of dinoflagellates larger than 20 μm was below 1% near the Marquesas Islands, 1% in the upwelling zone, 2% in the HNLC zone and around the station EGY, and reached a maximum of 3% at station ST20 probably because of the bloom observed there by Gomez et al. (2007).

From a global point of view, the abundance of dinoflagellates (Fig. 10) decreased towards the hyper-oligotrophic zone and increased towards the mesotrophic zone in agreement with Leterme et al. (2006) who showed that the dinoflagellate abundances increased with trophic status in the NE Atlantic Ocean. The increase in heterotrophic dinoflagellates contribution with depth that we observed is coherent with previous observation in the Equatorial Pacific (Chavez et al., 1990) Heterotrophic dinoflagellates were always much more abundant than ciliates as shown previously in the Sargasso Sea (Lessard and Murrell, 1996), and in the North-East Equatorial Pacific (Yang et al., 2004) and could be major predators of picoplankton (Sanders et al., 2000; Sherr et al., 1991).

Green fluorescing dinoflagellates were initially observed by Shapiro et al. (1989)

in the North-West Atlantic, but little reported since then. These authors found that they could contribute from 4 to 100% to heterotrophic dinoflagellates while Chavez et al. (1990) found that in the Equatorial Pacific, 32% of heterotrophic dinoflagellates produced a bright green fluorescence. The data reported here (maximal concentrations in excess of 60 cell mL^{-1} and maximum contribution up to 50% of the heterotrophic dinoflagellates, Fig. 10) are in agreement with these previous studies. (Shapiro et al., 1989). Still the origin of this bright green fluorescence (Fig. 4c) remains intriguing. Shapiro et al. (1989) hypothesized that it could be due to a flavoprotein. Kim et al. (2004) showed that the infection of the thecate dinoflagellate *Gonyaulax spinifera* by *Amoebophrya*, a parasitic dinoflagellate, induces a bright green autofluorescence iin infected cells. This fluorescence is, however, much more localised than in the green dinoflagellates observed in our samples (Fig. 4c). Another exciting possibility could be the presence of a cytoplasmic green fluorescing protein (Wilson and Hastings, 1998).

Ciliate abundances reported here (Table 1) are comparable to those reported from other similar marine systems ranging from oligotrophic to eutrophic (Beers et al., 1980; Leakey et al., 1996; Lessard and Murrell, 1996; Yang et al., 2004). Looking only at tintinnid ciliates, Dolan et al. (2007) observed during the BIOSOPE cruise much lower concentrations ranging from 0.002 and 0.04 cells mL^{-1} between 5 and 300 m. However, tintinnids generally account only for 5–10% of all ciliates (Dolan and Marrase, 1995). Comparing our data with values given in the Table 2 of Dolan et al. (2007) results in a proportion of tintinnids (0.05%) smaller than the one observed in the Catalan Sea (Dolan and Marrase, 1995). However, maxima and minima of tintinnid and total ciliates occur at the same place.

The distribution pattern of ciliates (Fig. 12). agrees with previous observations in the North Western Indian Ocean (Leakey et al., 1996) where the lowest abundances were observed in oligotrophic waters, and the highest in the most productive waters. The different patterns of vertical distribution of ciliates observed in the present study could be explained by the fact that no distinction has been made between the different types of ciliates (mixotrophic and heterotrophic ciliates). In the Catalan Sea, heterotrophic

ciliate distribution has been shown to be closely related to the DCM while mixotrophic ciliates have a more complicated vertical pattern and a distribution which may vary from system to system (Dolan and Marrase, 1995).

Nano-ciliates (<20 μm) have been identified as potentially important grazers of picoplankton (Sherr and Sherr, 1987; Sherr et al., 1991) and account in general from 50 to 95% of total ciliates in a variety of marine ecosystems (Beers et al., 1980; Montagnes et al., 1988; Yang et al., 2004). In our study however, we observed very few nanociliates since the size if the majority of ciliates felled into a 50–100 μm range (data not shown). This absence of nanociliates could be explained by our fixation method used in our study. Leakey et al. (1994) demonstrated that the use of glutaraldehyde as fixative could lead up to a loss of cells as high as 70% among aloricate ciliates relative to lugol's iodine while tintinnid numbers did not vary significantly between fixative treatments.

In conclusion, although assessing the abundance of the different microbial groups by microscopy is slow and labour-intensive, the present data set highlights some characteristics of the microbial community in the South East Pacific Ocean that have escaped more rapid techniques such as flow cytometry. This includes in particular the importance of colonial picocyanobacteria in the HNLC area and the large contribution of green fluorescing dinoflagellates in some regions, such between the gyre and the coast of South America.

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Table 1. Concentrations of the different populations enumerated in the present study. Average for the six depths sampled at each station.

Station	Latitude-Longitude	Picocyanobacteria containing phycoerythrin mL^{-1}	Total eukaryotes mL^{-1}	Autotrophic eukaryotes mL^{-1}	Heterotrophic eukaryotes mL^{-1}	Total dinoflagellates mL^{-1}	Autotrophic dinoflagellates mL^{-1}	Heterotrophic dinoflagellates mL^{-1}	Green dinoflagellates mL^{-1}
MAR1	08°23.464 S–141mL^{-1}14.364 W	3486.7	1520.8	1292.2	228.6	105.1	56.8	48.3	4.6
HLN1	09°00.543 S–136°51.025 W	2818.2	2312.2	1836.1	476.1	93.0	61.2	31.8	4.2
STB1	11°44.903,S–134°06.072 W	1612.3	1894.5	1164.8	729.7	111.1	61.7	49.5	4.5
STB3	15°31.981 S–129°55.376 W	412.7	1423.4	737.2	686.2	58.9	27.6	31.3	4.2
STB4	17°13.954 S–127°58.496 W	374.2	1266.7	735.9	530.8	57.4	25.6	31.8	7.0
STB6	20°26.632 S–122°54.812 W	6.2	1413.4	726.0	687.4	36.8	19.4	17.4	2.2
STB8	23°32.499 S–117°52.419 W	37.3	937.3	520.9	416.4	31.3	11.9	19.4	3.5
GYR2	25°58.746 S–114°00.37 W	46.0	805.5	540.8	264.8	42.5	20.9	21.6	3.5
STB11	27°45.823 S–107°16.367 W	33.6	1050.4	525.8	524.6	31.1	10.2	20.9	6.5
STB14	30°02.050 S–098°23.623 W	141.7	1314.0	854.0	460.0	54.9	22.4	32.6	8.5
EGY2	31°50.575 S–091°27.684 W	1734.2	3082.9	2481.3	601.7	81.8	47.0	34.8	6.5
STB17	32°23.706 S–086°47.165 W	1103.9	2606.8	2086.0	520.9	94.0	45.7	48.2	12.4
STB20	33°21.365 S–078°06.180 W	10725.7	1760.3	1194.6	565.6	92.2	44.3	48.0	6.7
UPW1	34°01.196 S–073°21.511 W	40548.1	3396.2	2526.0	870.2	121.8	63.2	58.7	14.9
UPX2	34°37.975 S–072°27.818 W	18548.4	14088.1	12211.4	1876.6	151.0	46.5	104.4	37.6

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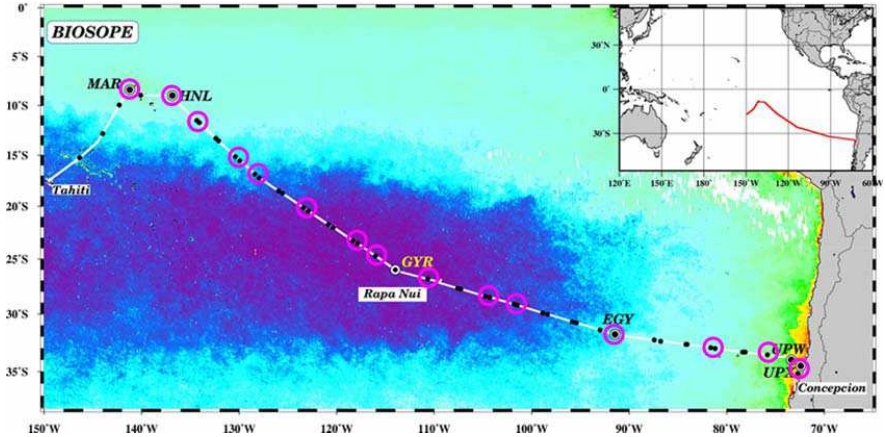

Fig. 1. Map of the BIOSOPE cruise track superimposed on a SeaWiFS ocean colour composite, the dark purple indicating extremely low values (0.018 mg m^{-3}) of total chlorophyll *a*. Pink circles indicate the stations analysed by DAPI staining in this study.

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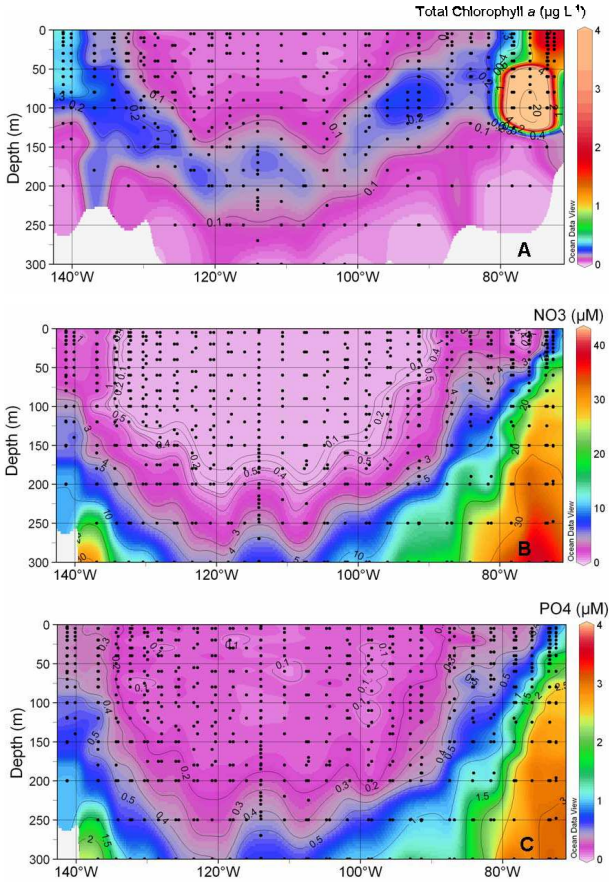

Fig. 2. Distribution with longitude and depth of total chlorophyll a (Tchl*a*, chlorophyll *a* + divinyl chlorophyll *a*) in $\mu g\,L^{-1}$ (Ras et al., 2007[4]) **(A)**, nitrate in μM (Raimbault and Garcia, 2007[2]) **(B)**, phosphate in μM (Moutin et al., 2007[3]) **(C)**.

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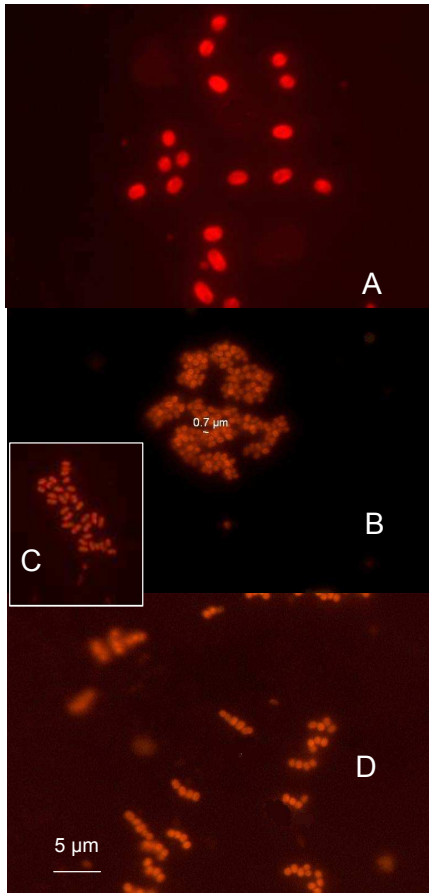

Fig. 3. Pictures of single **(A)**, and colonial picocyanobacteria (**B**–**D**). Colony of more than 100 cells (B), colony of 20–30 cells **(C)**, Chain forming cells (D). Pictures have been taken under green light excitation on samples of stations MAR1 at 80 m (A), MAR1 at 40 m (B), HNL1 at 60 m (C), and STB3 at 60 m (D).

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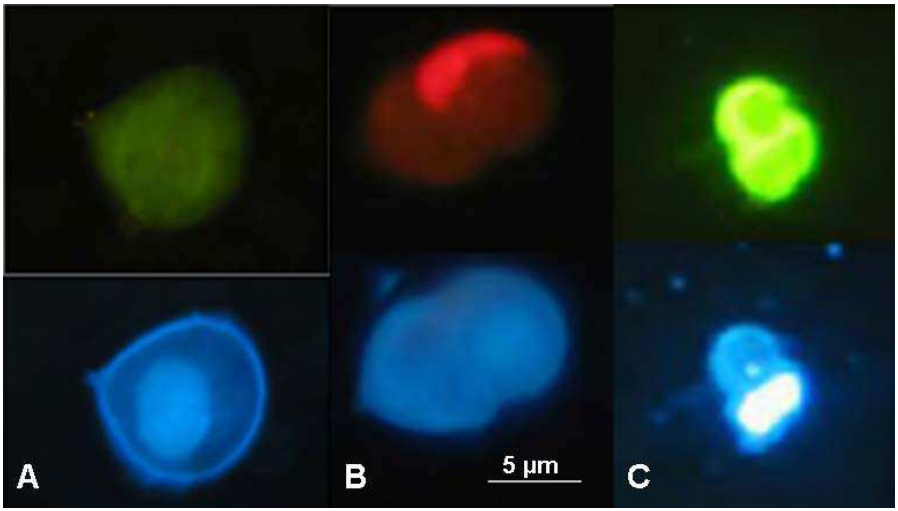

Fig. 4. Heterotrophic **(A)**), autotrophic **(B)**, and green dinoflagellates **(C)** observed under blue light excitation (top) and UV light excitation (bottom). Pictures have been taken at stations STB3 (20 m), UPW and STB7 (5 m), respectively.

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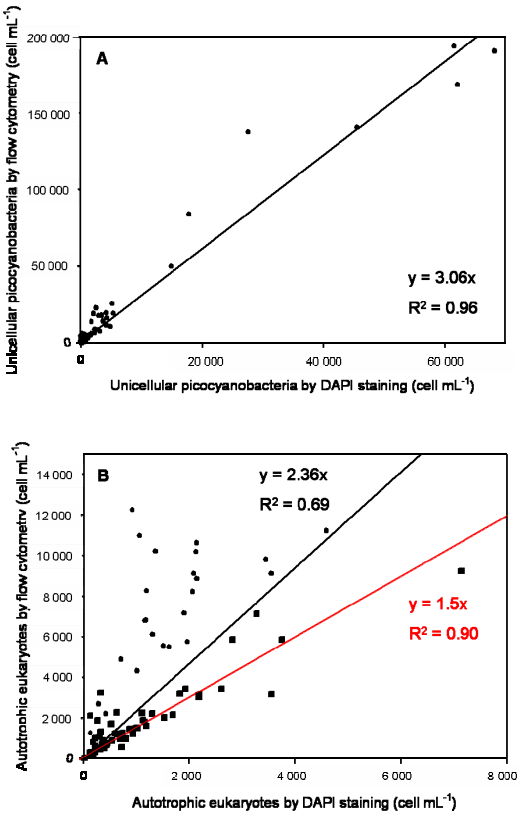

Fig. 5. Relationship between the abundances (cell mL^{-1}) measured by flow cytometry (Grob et al., 2007) and those estimated by DAPI counting of unicellular cyanobacteria **(A)**, and autotrophic eukaryotes **(B)**. (A) R^2=0.96, n=80; (B) Regression line in black takes into account all data (circles and squares); R^2=0.69 n=80. Regression line in red takes into account only square dots; R^2=0.90, n=56.

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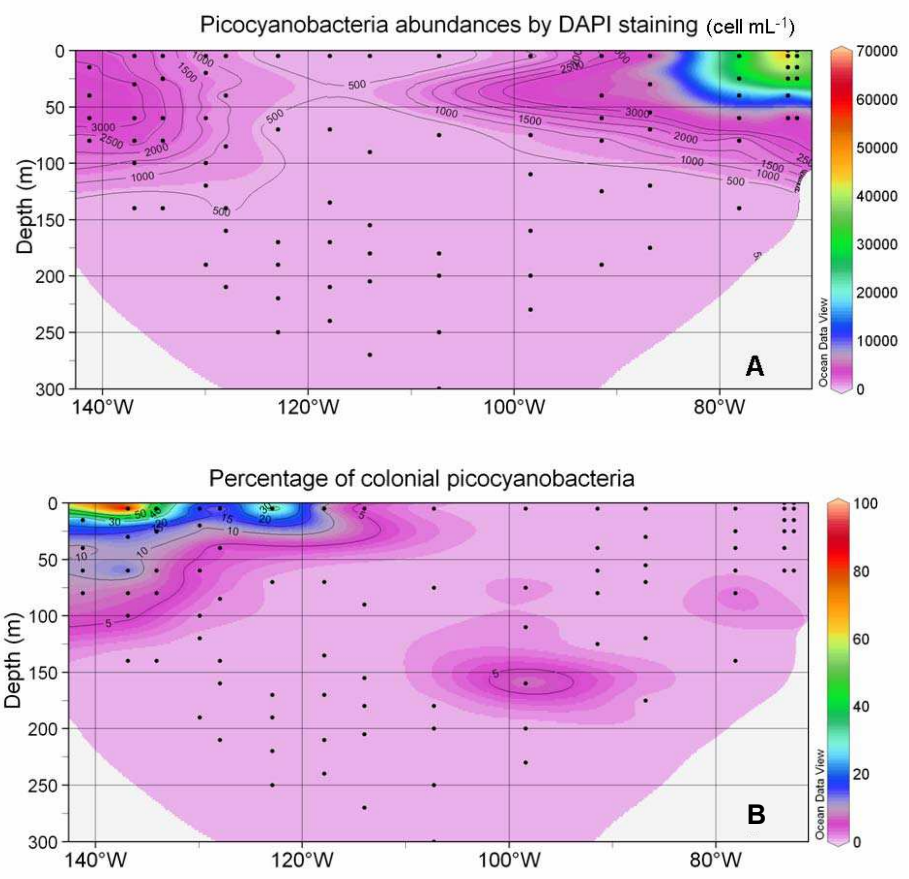

Fig. 6. Distribution of abundances obtained by DAPI counting for unicellular picocyanobacteria (cell mL^{-1}) **(A)** and percentage of unicellular picocyanobacteria in colony **(B)**. Black dots correspond to the samples analysed. Contour plots generated with the software Ocean Data View.

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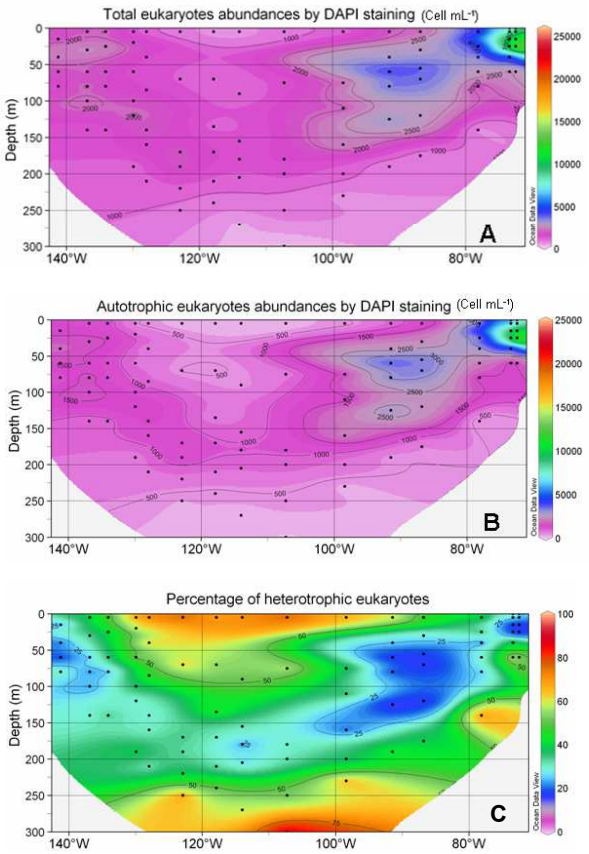

Fig. 7. Distribution obtained by DAPI counting of total eukaryotes (cell mL^{-1}) **(A)**, autotrophic eukaryotes (cell mL^{-1}) **(B)**, and percentage of heterotrophic eukaryotes in comparison with total eukaryotes **(C)**. Legend as in Fig. 6.

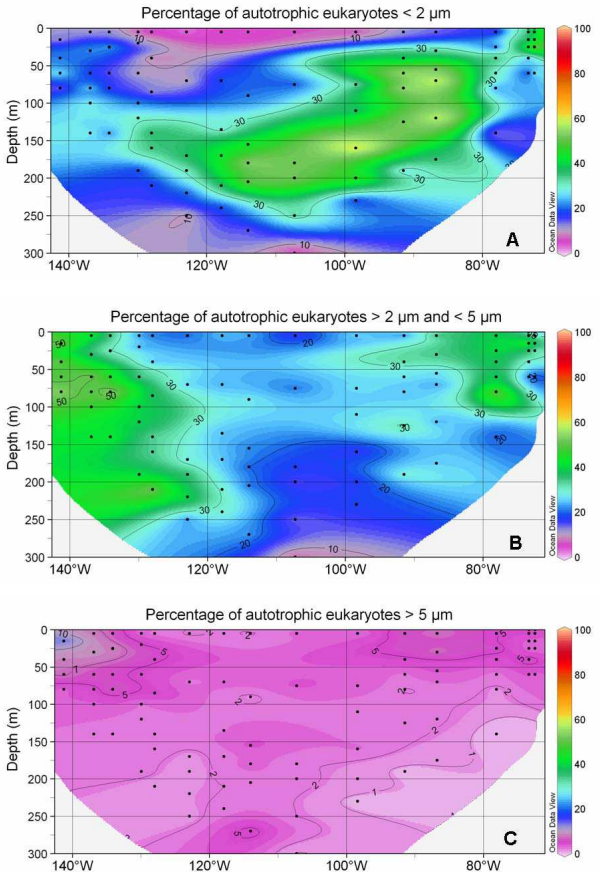

Fig. 8. Distribution of the percentage of autotrophic eukaryotes smaller than 2 μm **(A)**, between 2 μm and 5 μm **(B)**, and larger than 5 μm **(C)** in comparison with the total eukaryotes. Legend as in Fig. 6.

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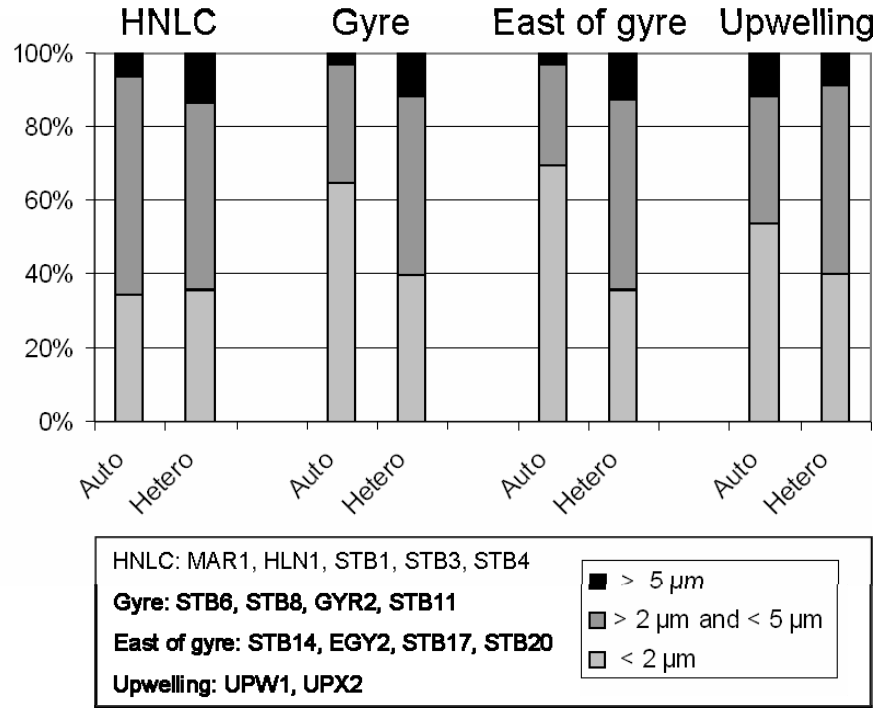

Fig. 9. Contribution of the different size classes for the autotrophic (Auto) and heterotrophic (Hetero) eukaryotes at the depth of chlorophyll maximum for the HNLC, Gyre, East of gyre and Chile upwelling regions.

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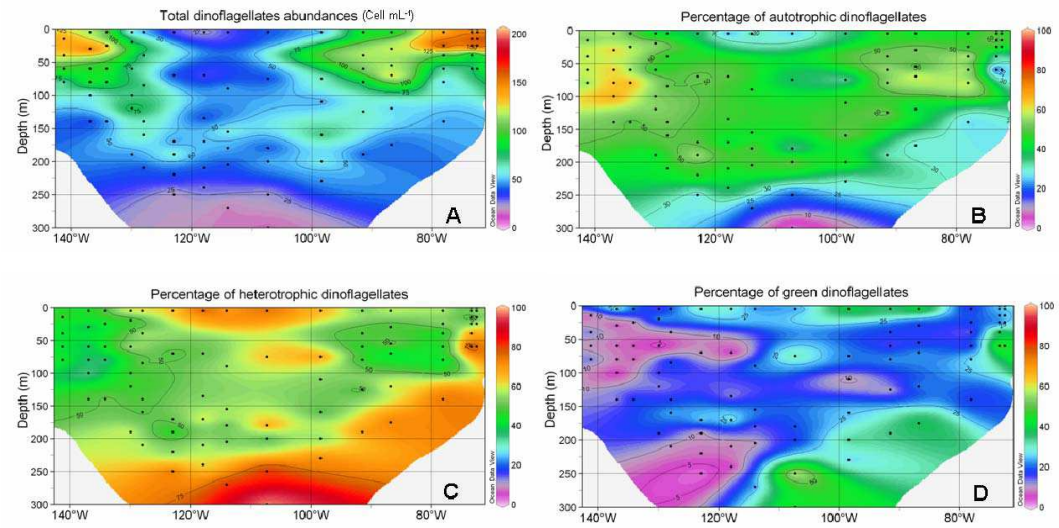

Fig. 10. Distribution of total dinoflagellates (cell mL^{-1}) **(A)**, percentage of autotrophic dinoflagellates **(B)**, percentage of heterotrophic over total dinoflagellates **(C)**, and percentage of green over total heterotrophic dinoflagellates **(D)**. Legend as in Fig. 6.

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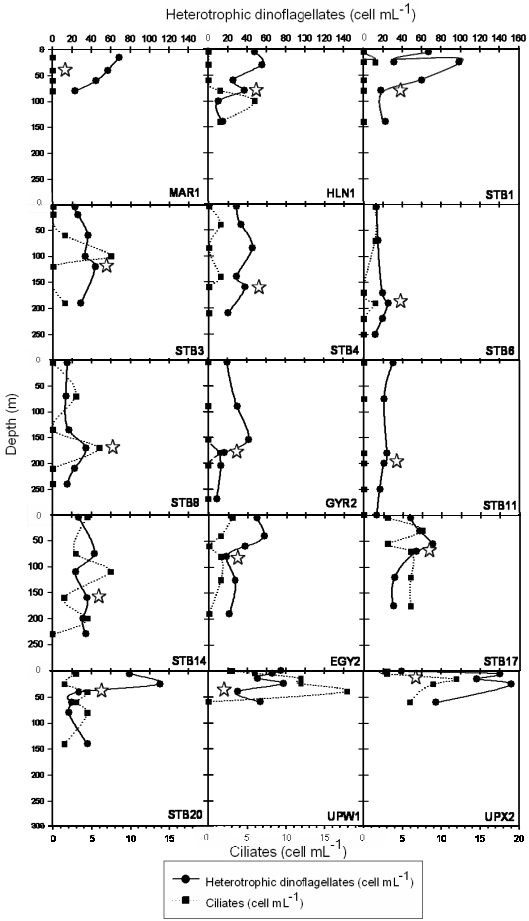

Fig. 11. Vertical profiles of concentrations (cell mL^{-1}) of total heterotrophic dinoflagellates (solid line) and ciliates (dotted line). Stars indicate the depth of chlorophyll maximum.

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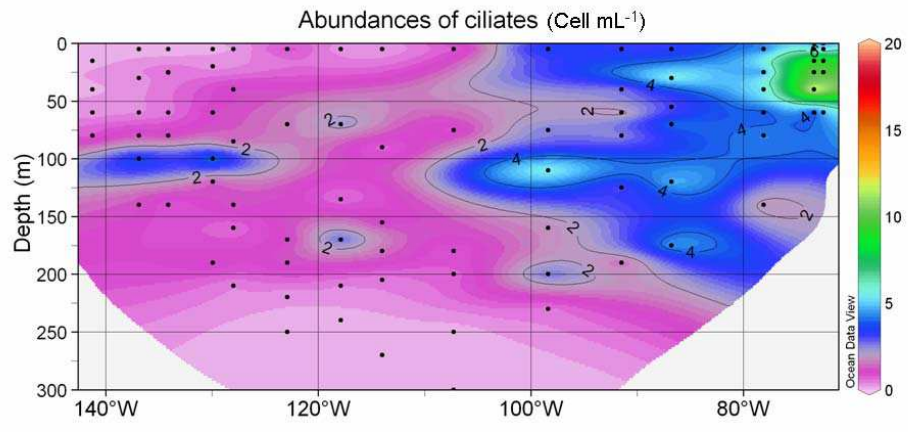

Fig. 12. Distribution of ciliates (cell mL^{-1}). Legend as in Fig. 6.

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